

# Multiple Osteochondromas: Clinicopathological and Genetic Spectrum and Suggestions for Clinical Management

Liesbeth Hameetman<sup>1</sup>, Judith V.M.G. Bovée<sup>1</sup>, Antonie H.M. Taminiau<sup>2</sup>, Herman M. Kroon<sup>3</sup>, Pancras C.W. Hogendoorn<sup>1</sup>

<sup>1</sup>Departments of Pathology, <sup>2</sup>Orthopaedic Surgery and <sup>3</sup>Radiology, Leiden University Medical Centre, Leiden, The Netherlands

**Key words:** bone neoplasm, multiple osteochondromas, genetics, clinical management, chondrosarcoma, exostosis

Corresponding author: P.C.W. Hogendoorn, PhD MD, Department of Pathology, Leiden University Medical Centre, L1-Q, PO Box 9600, 2300 RC Leiden, The Netherlands (e-mail: p.c.w.hogendoorn@lumc.nl).

Submitted: 18 August 2004

Accepted: 15 November 2004

## Abstract

Multiple Osteochondromas is an autosomal dominant disorder characterised by the presence of multiple osteochondromas and a variety of orthopaedic deformities. Two genes causative of Multiple Osteochondromas, *Exostosin-1* (EXT1) and *Exostosin-2* (EXT2), have been identified, which act as tumour suppressor genes. Osteochondroma can progress towards its malignant counterpart, secondary peripheral chondrosarcoma and therefore adequate follow-up of Multiple Osteochondroma patients is important in order to detect malignant transformation early.

This review summarizes the considerable recent basic scientific and clinical understanding resulting in a multi-step genetic model for peripheral cartilaginous tumorigenesis. This enabled us to suggest guidelines for clinical management of Multiple Osteochondroma patients. When a patient is suspected to have Multiple Osteochondroma, the radiologic documentation, histology and patient history have to be carefully reviewed, preferably by experts and if indicated for Multiple Osteochondromas, peripheral blood of the patient can be screened for germline mutations in either *EXT1* or *EXT2*. After the Multiple Osteochondroma diagnosis is established and all tumours are identified, a regular follow-up including plain radiographs and base-line bone scan are recommended.

## Introduction

Osteochondroma is the most common benign bone tumour, which occurs as sporadic (solitary) or multiple, usually in the context of the hereditary syndrome, Multiple Osteochondromas (MO) [1, 2]. Considerable understanding obtained through research on the genetic, pathological and radiologic background of these tumours, has provided insights into the tumorigenesis of Multiple Osteochondromas resulting in the optimisation of clinical management, including radiologic and mutational screening.

## Incidence

Osteochondromas represent about 50% of all surgically treated primary benign bone tumours [1]. Approximately 15% of the osteochondroma patients have multiple lesions [1, 3] of which 62% have a positive family history [4].

The incidence for Multiple Osteochondromas has been estimated at 1:50,000 in the general population [5], with a higher prevalence in males (male:female ratio of 1.5:1) [4, 6], which is partly due to incomplete penetrance in females [4].

## Osteochondroma

Osteochondroma (*osteocartilaginous exostosis*), according to the 2002 WHO definition, is a cartilage capped benign bony neoplasm on the outer surface of bones preformed by endochondral ossification [7-9]. They develop and increase in size in the first decade of life and cease to grow at skeletal maturation or shortly thereafter. The most common site of involvement is the metaphyseal region of the long bones of the limbs, like the distal femur, upper humerus, upper tibia and fibula [1, 8]. However, osteochondromas also occur in flat bones, in particular the ilium and scapula. An important differential diagnostic feature as compared to e.g. metachondromatosis or parosteal and periosteal osteosarcoma, is the extension of the medullar cavity into the lesion and the continuity of the cortex with the underlying bone. The perichondrium, the outer layer of osteochondroma, is continuous with the periosteum of the underlying bone.

Many osteochondromas are cauliflower shaped and can be divided on macroscopical grounds to often long slender pedunculated osteochondromas and flat sessile ones.

In the cartilage cap the chondrocytes are arranged in a similar fashion as in the epiphyseal growth plate. As a typical benign tumour the chondrocytes have small single nuclei. Binucleated chondrocytes may be seen during active growth.

The stalk may fracture, which may result in reactive fibroblastic proliferation and new bone formation, erroneously leading to interpretation as the formation of secondary sarcoma. Attached to the perichondrium a secondary bursa may develop and simulate the growth of the underlying tumour. This bursa is lined by synovium and may show inflammatory changes [3].

## Multiple Osteochondromas

Multiple Osteochondromas (hereditary multiple exostoses, diaphyseal aclasis) are characterised by the presence of multiple osteochondromas [2, 4, 6, 10, 11] the number of which can vary significantly between and within families. Most Multiple Osteochondroma patients also suffer from a variety of orthopaedic deformities like shortening of the ulna with secondary bowing of the radius (39-60%), inequality of the limbs (10-50%), varus or valgus angulation of the knee (8-33%), deformity of the ankle (2-54%) and disproportionately short stature [2, 4-6, 12]. It has been a matter of debate whether these deformities are a result of skeletal dysplasia or a result of local effects on the adjacent growth plate caused by developing osteochondromas.

No well-documented association between Multiple Osteochondromas and other non-bone related disorders has been described so far.

## Malignant transformation

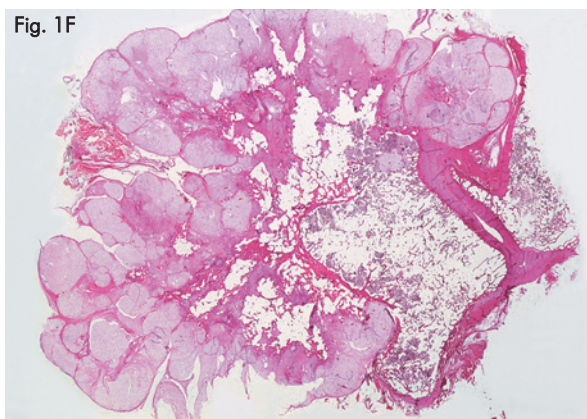
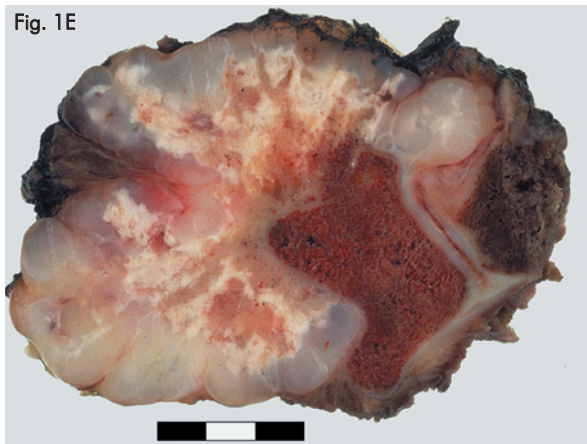
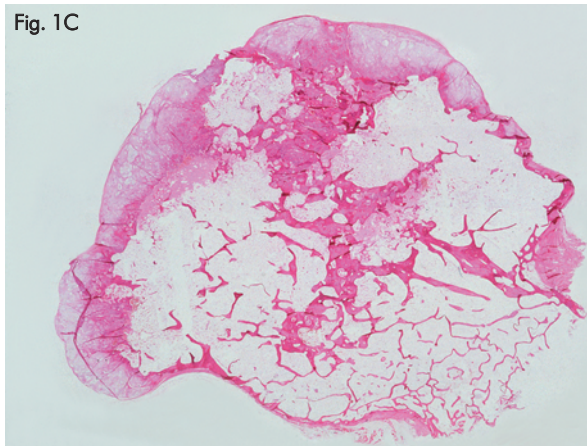
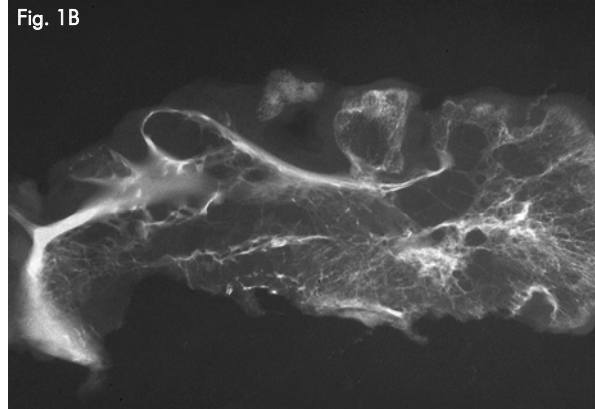
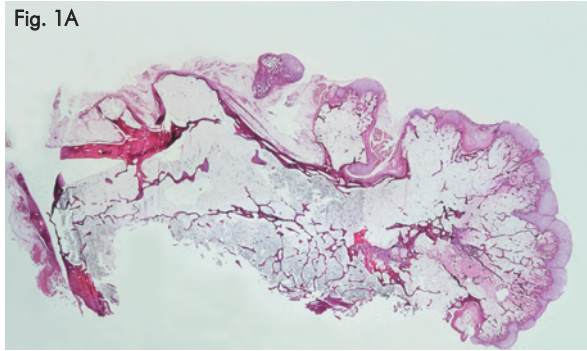
Malignant transformation of osteochondroma is estimated to be less than 1% in patients with solitary lesions and 0.5-3% in patients with Multiple Osteochondromas [2, 7]. In 94% of the cases with malignant progression a secondary peripheral chondrosarcoma has developed within the cartilage cap of an osteochondroma [13]. Secondary peripheral chondrosarcoma is a hyaline cartilage producing tumour and constitutes approximately 15% of all chondrosarcomas [1, 14], which is the third most frequent malignant bone tumour after myeloma and osteosarcoma [15]. Increasing pain, functional disability and/or a growing mass, specifically after maturation of the skeleton, may indicate malignant transformation. Radiologic features show irregular mineralisation and increased thickness (over 2 cm) of the cartilage cap of an osteochondroma. The cap shows lobules of hyaline cartilage that are separated by bands of fibrous tissue [15]. With (dynamic) contrast enhanced magnetic resonance (MR) imaging this can be seen as septal enhancement whereas osteochondromas only display peripheral enhancement. High-grade peripheral chondrosarcomas are characterised by inhomogeneous and homogeneous enhancement patterns on gadolinium-enhanced MR images [16, 17].

The histological grading of chondrosarcoma is based on nuclear size and chromasia and cellularity [18] and is the most important predictor of clinical behaviour and thus prognosis of patients with chondrosarcomas [15]. Chondrosarcomas secondary to osteochondromas are usually low-grade tumours resulting in a reasonably fair prognosis for these patients [15].

In the remaining 6% of the cases with malignant progression tumours arise in the bony stalk of the osteochondroma, including osteosarcomas and spindle cell sarcomas [19-22].

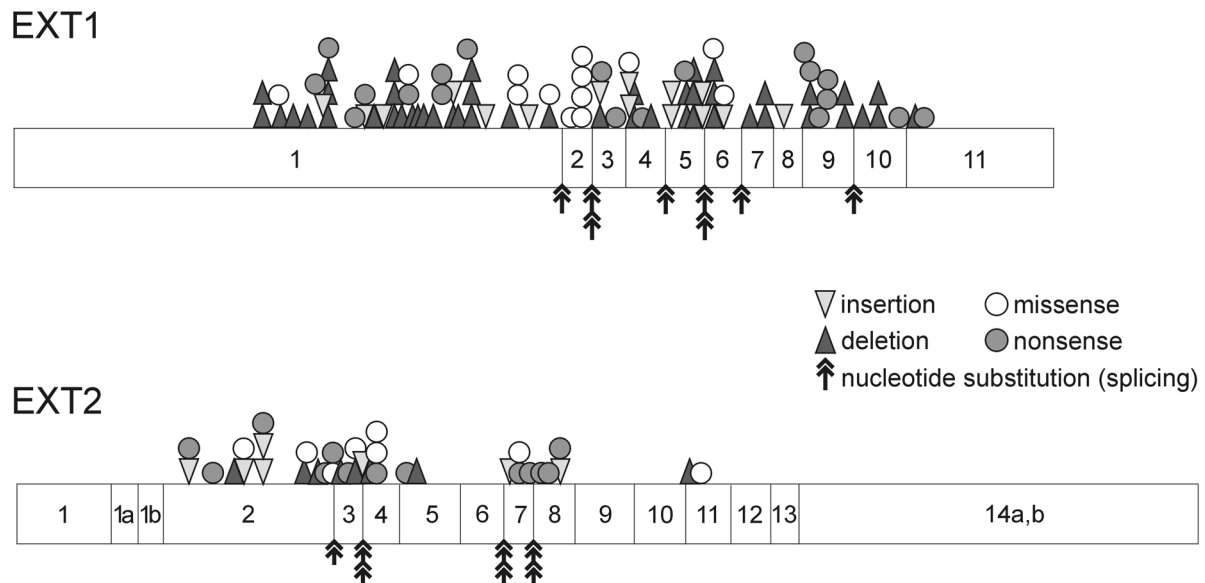
## Genetics

Multiple Osteochondromas is an autosomal dominant disorder for which two genes have been isolated, *Exostosin-1* (*EXT1*; OMIM 133700) located at 8q24 and *Exostosin-2* (*EXT2*; OMIM 133701) located at 11p11-p12 [23-25]. 44-66% of the Multiple Osteochondroma families show linkage at the *EXT1* region [26, 27], compared to 27% for *EXT2* [27]. Germline mutations of *EXT1* and *EXT2* have been described in Multiple



**Fig. 1.** Specimen radiographs and histology

A pedunculated osteochondroma shown in a macroscopic whole mount section (**A**) and specimen radiograph (**B**); **C**, whole mount section of a sessile osteochondroma. Note the presence of a small cartilage cap in both osteochondromas (<0.5 cm); **D**, radiograph of the forearm of a Multiple Osteochondroma patient. Several osteochondromas can be seen at the ends of the ulna and radius. Note that the ulna is shortened, which caused subsequent bowing of the radius; **E** and **F**, gross specimen and whole mount section of secondary peripheral chondrosarcoma. The cartilage cap is thicker than 2 cm and in the whole mount section the lobules are clearly visible.



**Fig. 2.** Mutation spectrum of the *EXT1* and *EXT2* genes in MO patients described so far [106]

Osteochondroma patients from Caucasian [23, 25, 28-31] and Asian populations [32-34].

Most mutations (80%) found in *EXT1* and *EXT2* (Fig. 2) are either nonsense, frameshift or splice-site mutations leading to premature terminations of the *EXT* proteins (reviewed by Zak et al [35]). Mutations in *EXT1* occur in all parts of the gene, while mutations in *EXT2* concentrate towards the N-terminus of the gene, implying that this part of the protein may have special functions. This seems contradictory, since only the C-terminal region is highly conserved, implicating some functional importance for this part of the protein [24, 25]. In the literature, only one somatic mutation in the *EXT1* gene has been described in a sporadic chondrosarcoma [29].

Loss of the remaining wild-type allele has been demonstrated in hereditary osteochondromas [31], indicating that the *EXT* genes act as tumour suppressor genes in Multiple Osteochondromas. This is consistent with Knudson's two-hit model for tumour suppressor genes [36].

Not many genotype-phenotype correlation studies have been described to draw definitive conclusions [37, 38]. There seems to be a slightly higher risk of malignant transformation in patients with an *EXT1* mutation as compared to *EXT2* [38].

The existence of a third *EXT* gene on chromosome 19p, *EXT3* [39], has been suggested, however no gene has been identified, nor has this locus been implicated by other researchers.

Based on their homology with *EXT1* and *EXT2*, three other members of the *EXT*-family of genes, the

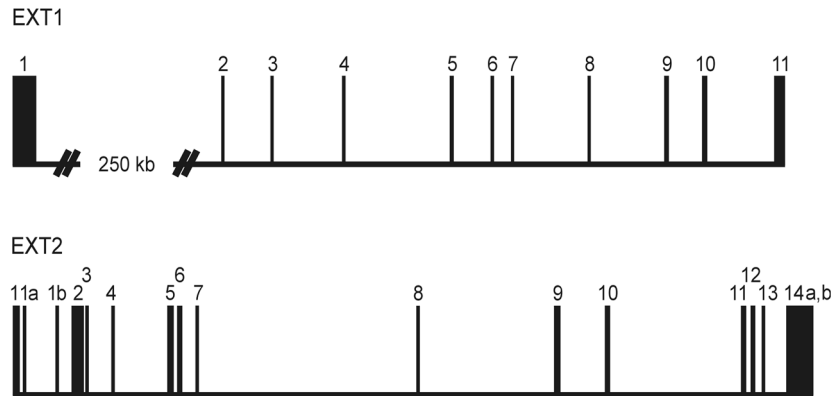
*EXT*-like genes (*EXTL1-3*), have been identified [40-42]. *EXTL1*, *EXTL2* and *EXTL3* are located at 1p36.1 [40], 1p11-p12 [41] and 8p12-p22 [42], respectively. No linkage with Multiple Osteochondromas or other bone diseases has been documented for these genes [43].

## EXT1

Before linkage to Multiple Osteochondromas, osteochondromas were already known to be involved in a contiguous gene deletion syndrome, the Langer Gideon syndrome (LGS or trichorhinophalangeal syndrome type II; OMIM150230) [44], where patients carry a deletion of 8q24 [45]. Besides multiple osteochondromas the Langer Gideon syndrome is characterised by craniofacial dysmorphism and mental retardation [44, 45].

In the early nineties Cook et al found linkage to the 8q24.11-q24.13 region in Multiple Osteochondroma families [46] and two years later the *EXT1* gene was identified by positional cloning [23].

The *EXT1* gene, composed of 11 exons, spans approximately 350kb of genomic DNA (Fig. 3) [47] with a promoter region that has the characteristics of a house keeping gene [47]. *EXT1* mRNA is ubiquitously expressed and has a coding sequence of 2238 bp [23]. In mouse embryos, high mRNA levels of the *EXT1* homologue have been found in the developing limb buds [48,49]. *EXT1* homologues have also been identified in *Drosophila melanogaster* (*tout-velu*, *Ttv*) and *Caenorhabditis elegans* [50, 51].



**Fig. 3.** Genomic DNA structures of the EXT1 and EXT2 genes

## EXT2

In two large Multiple Osteochondroma pedigrees not linked to 8q24, linkage was found to a 3 cM region located at 11p11-p12, excluding the pericentromeric region [52, 53]. In 1996, the *EXT2* gene was identified by positional cloning by two groups independently [24, 25].

The *EXT2* gene contains 16 exons (Fig. 3) and spans approximately 108 kb of genomic DNA [51]. The mRNA consists of approximately 3kb, with a single open reading frame of 2154 bp in which the C-terminal region shows high similarity with *EXT1* [24, 25]. The mRNA shows alternative splicing in exon 1a and 1b and is ubiquitously expressed [24, 25]. Homologues of *EXT2* have been found in mouse (chromosome 2) [51, 54], *Drosophila melanogaster* (*sister of tout-velu*, *sotv*) [55] and *Caenorhabditis elegans* [51].

Like *EXT1*, *EXT2* has been implicated in a contiguous gene deletion syndrome, Potocki-Shaffer syndrome (DEFECT11; OMIM 601224), where patients carry a deletion of 11p11.2-p12 [56, 57]. Patients with this syndrome demonstrate multiple osteochondromas, enlarged parietal foramina (FPP), craniofacial dysostosis and mental retardation [56, 57].

## EXT function

The gene products of human *EXT1* and *EXT2* are endoplasmic reticulum localised type II transmembrane glycoproteins. In vivo they form a stable hetero-oligomeric complex that accumulates in the Golgi apparatus, where it is involved in heparan sulphate proteoglycan (HSPG) biosynthesis (reviewed by Esko et al [58]) (Fig. 4). The *EXT1/EXT2* complex catalyses the elongation of the HS chain [59-62], which is

subsequently deacetylated, sulphated and epimerised resulting in a large spectrum of structural heterogenic HS chains. The sulphation pattern of HS chains is critical for binding specific proteins [58]. Several growth factors have conserved patterns of basic amino acids for binding to HSPGs, which is crucial for proper signalling [63, 64].

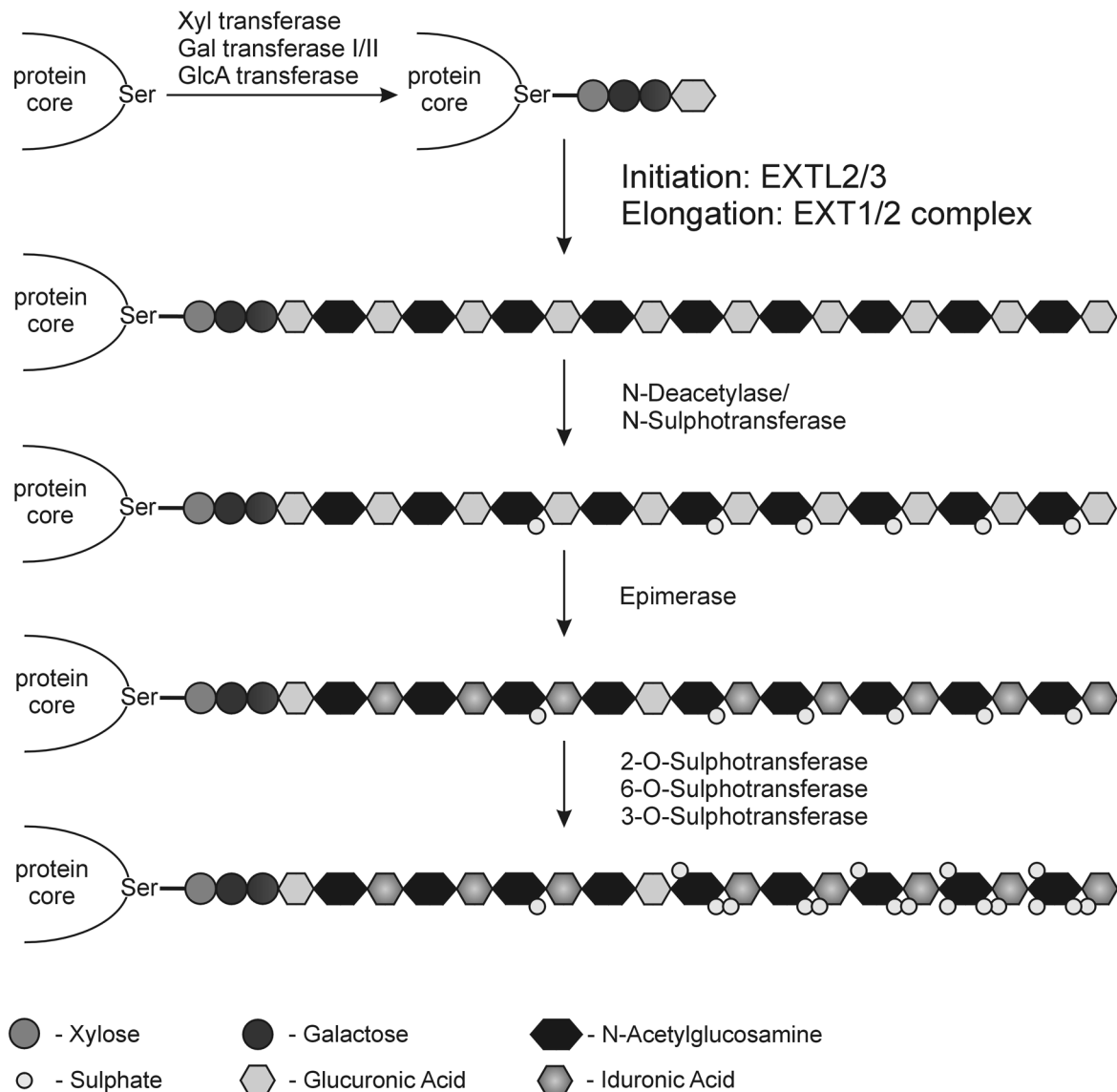
## Heparan Sulphate Proteoglycans (HSPG)

HSPGs are large multifunctional macromolecules involved in several growth signalling pathways, anchorage to the extracellular matrix and sequestering of growth factors (reviewed by Knudson [65]). Four HSPG families have been identified: syndecan, glypican, perlecan and CD44 isoforms.

The syndecan family consists of four members, encoding type I transmembrane polypeptides involved in the anchorage of cells to the extracellular matrix and binding of growth factors [66]. In mouse and chick, syndecan-2 and -3 have shown to be involved in signalling pathways in proliferating chondrocytes [67-70].

The six glypican family members encode proteins attached to the cell membrane with a glycosylphosphatidylinositol (GPI)-anchor. They predominantly function as co-receptors [66]. Expression of several glypicans has been found in the perichondrium, the developing limb and mesenchymal tissues of the developing mouse embryo [71].

The largest HSPG, perlecan, is the most common proteoglycan of the basement membrane. It is expressed in hyaline cartilage and in all zones of the rat growth plate during endochondral ossification [72]. Perlecan, syndecan and glypican are reported to be involved in FGF-signalling [65, 66].



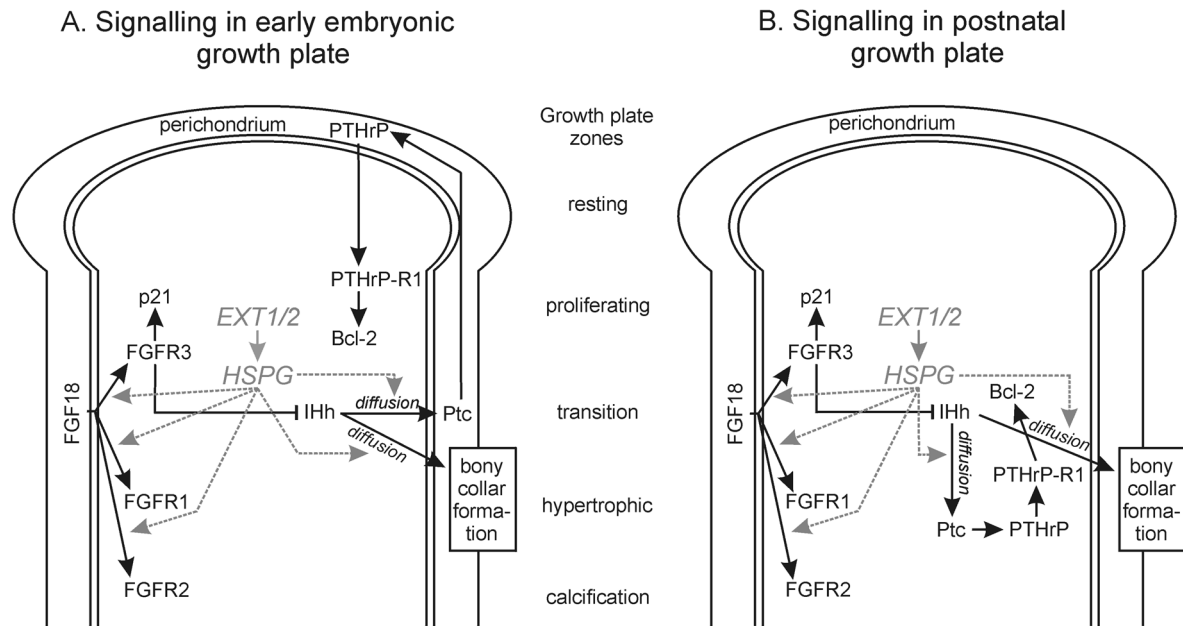
**Fig. 4.** The mode of action of the EXT-proteins in heparan sulphate biosynthesis

After a tetrasaccharide linker is synthesised on conserved serine residues of the core protein, EXTL2 and/or EXTL3 initiate the polymerisation of the heparan sulphate chain by the addition of N-acetylglucosamine [60, 107]. The EXT1/EXT2 complex subsequently catalyses further elongation of the heparan sulphate chain by adding alternating units of N-acetylglucosamine and glucuronic acid [59-62]. Subsequent deacetylation and sulphation of most N-acetylglucosamines, epimerisation of the glucuronic into iduronic acid and further sulphation result in a large spectrum of structural heterogenic heparan sulphate chains [58, 108].

Adapted from Couchman et al [109] and Nybakken et al [110].

The fourth HSPG family is specific isoforms of the type I transmembrane glycoproteins CD44. The CD44 gene consists of 20 exons of which 10 (so-called variable exons) can be alternatively spliced (reviewed by Ponta et al [73]). CD44 isoforms containing variable exon 3 (v3) have been shown to bind growth factors through HS side chains, thereby regulating cell growth and motility [74].

In *Drosophila*, the *EXT1* homologue *Ttv* (*tout-velu*), also involved in HS synthesis, is required for the diffusion of Hedgehog (Hh), an important segment polarity protein (homologue of mammalian IHH) [50]. Remarkably, in *Ttv* mutants only the IHH signalling is affected, while other HSPG-dependent pathways, like FGF and WNT signalling, are not. This indicates a specificity in the regulation of the distribution of extracellular signals by HSPGs in *Drosophila* [75, 76].



**Fig. 5.** Growth plate signalling

EXT1 and EXT2 are expressed in the proliferative and transition zone [77] (Fig. 5). The HSPGs, expressed in all zones of the growth plate [67-72]. A. In the embryonic growth plate chondrocytes in the transition zone secrete IHh protein, which diffuses to its receptor Patched (Ptc) in the lateral perichondrium. Subsequently, via a yet incompletely understood mechanism, increased secretion of ParaThyroid Hormone related Protein (PTHrP, PTHIP) is induced at the apical perichondrium, which diffuses to its receptor expressed in the late proliferating chondrocytes [80]. Terminal differentiation is inhibited by direct or indirect upregulation of Bcl-2, prolonging cell survival [78]. In this way, PTHrP regulates chondrocyte differentiation by delaying the progression of chondrocytes towards the hypertrophic zone and allowing longitudinal bone growth. B. In the post-natal growth plate the signalling is confined to the growth plate [79].

## Growth Signalling

### Indian Hedgehog (Ihh)/PTHrP signalling in the growth plate

In the growth plate EXT1 and EXT2 are expressed in the proliferative and transition zone [77] (Fig. 5). The HSPGs, expressed in all zones of the growth plate [67-72], are presumed to be involved in the diffusion of IHh to its receptor in the perichondrium. During normal embryonic growth IHh, expressed in the transition zone, is involved in a paracrine feedback loop regulating proliferation and differentiation of chondrocytes and bony collar formation in the growth plate (Fig. 5A). In this feedback loop PTHrP regulates chondrocyte differentiation by delaying progression of chondrocytes towards the hypertrophic zone, allowing longitudinal bone growth [78]. In the rat post-natal growth plate the feedback loop is confined to the growth plate itself (Fig. 5B), in particular to the transition zone [79].

### Fibroblast Growth Factor (FGF) signalling in the growth plate

The FGF-signalling pathway is dependent on HSPGs for the high affinity binding capacity of the FGF receptor (FGFR), allowing receptor dimerisation and

subsequent cell signalling [80, 81]. The most potent mitogen for chondrocytes, FGF-2 (basic FGF), inhibits differentiation of chondrocytes via stimulation of extracellular matrix synthesis [82, 83]. In contrast, activation of FGFR3 in the proliferative zone (Fig. 5), by FGF18 [84] inhibits chondrocyte proliferation via phosphorylation of STAT-1 and subsequent upregulation of p21<sup>WAF/CIP1</sup>, which can inhibit the cell cycle [85]. FGFR3 activation also leads to repression of IHh signalling [80, 81, 86].

## Histogenesis and secondary sarcoma formation

In the past, many have considered the histogenesis of osteochondroma as a perversion in the direction of normal bone growth resulting from aberrant epiphyseal development with displacement of epiphyseal cartilage. However, several research groups have demonstrated using different techniques that both sporadic and hereditary osteochondromas are true neoplasms [31, 87, 88], resulting in a multi-step genetic model for peripheral cartilaginous tumorigenesis (Fig. 6) [89].

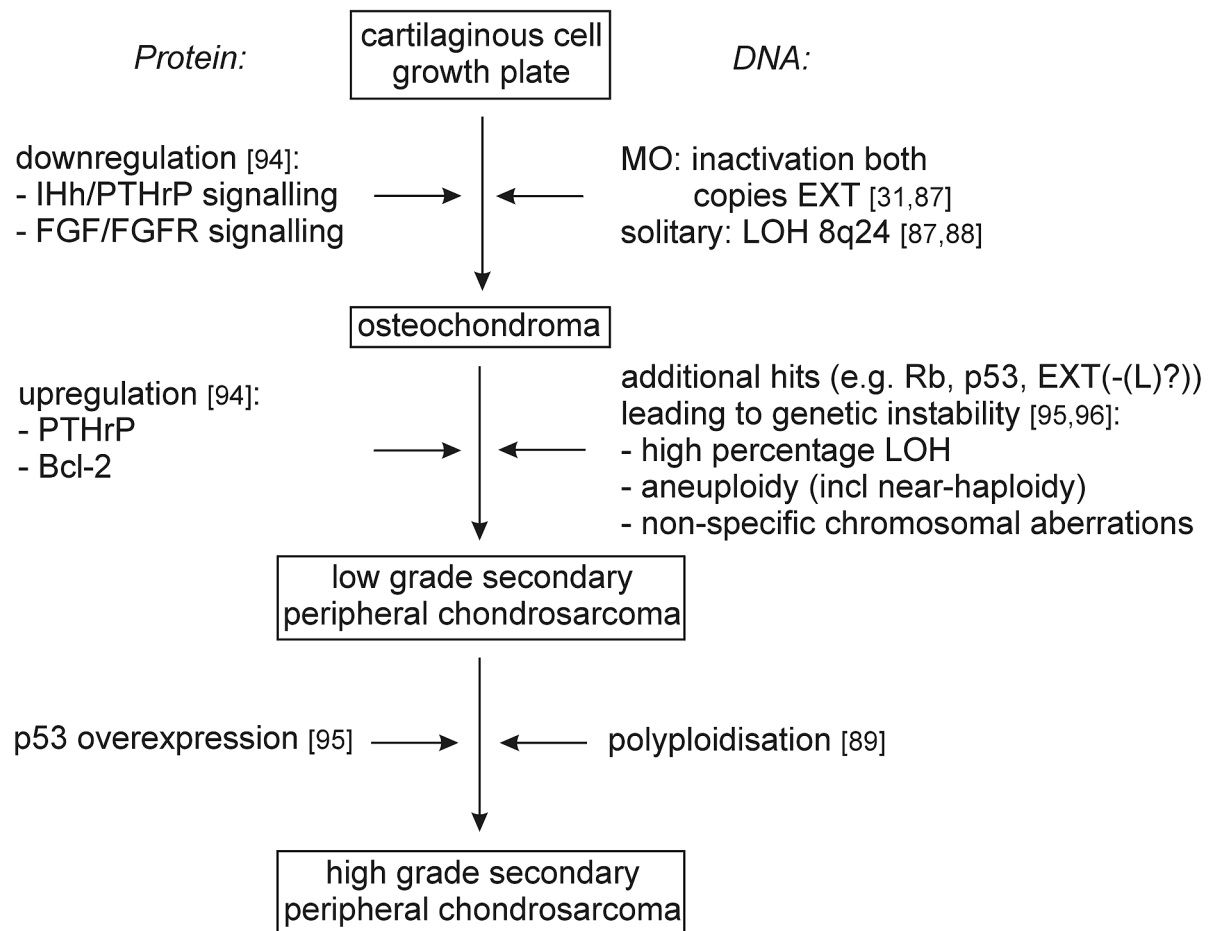


Fig. 6. Peripheral Cartilaginous Tumorigenesis

Although some believe that the severity of the angular deformity is correlated with the number of sessile osteochondromas [37], several studies in mice have shown that haploinsufficiency of *EXT1* or *EXT2* causes severe skeletal deformities [90, 91]. Loss of the remaining wild-type allele of *EXT1* in hereditary osteochondromas [31] indicated that inactivation of both copies of the *EXT1* gene in cartilaginous cells of the growth plate is required for osteochondroma formation, thereby acting as a tumour suppressor gene [31]. Two studies have shown diminished HSPG expression in either osteochondromas or cultured *EXT1*<sup>-/-</sup> cells [92, 93]. This is hypothesised to affect the negative feedback loop by disturbing Ihh diffusion to Ptc and by preventing high-affinity binding of FGF to its receptor (Fig. 5). Immunohistochemical studies have already shown that molecules involved in the Ihh/PTHrP and FGF/FGFR signalling (PTHrP, PTHrP-R1, Bcl-2, FGF2, FGFR1, FGFR3 and p21) are absent in

osteochondromas [94] suggesting that growth signalling is indeed disturbed in osteochondroma.

At the protein level, re-expression of several of these signalling molecules (FGF2, FGFR1, p21, PTHrP and Bcl-2) was found in secondary peripheral chondrosarcoma and the expression increased with increasing histological grade [94]. Upregulation of Bcl-2 characterised malignant transformation of osteochondroma towards grade I secondary peripheral chondrosarcoma [94]. Signalling may now occur in an autocrine fashion or in a paracrine one in which Ihh acts on cells in its near vicinity, having to diffuse over only a few cell diameters and thereby avoiding HSPG-dependent diffusion [94].

The process of malignant transformation is genetically represented by chromosomal instability [95], probably caused by defects in spindle formation. The LOH found in osteochondroma was restricted to 8q24 [31], whereas in secondary peripheral chondrosarcomas LOH was found in virtually all loci tested [95]. Also a



broad range in DNA ploidy including near-haploidy and non-specific chromosomal alterations were found [95, 96]. DNA-flow cytometry of the cartilaginous cap of osteochondromas showed mild aneuploidy [31], whereas more severe aneuploidy [97-99], including near-haploidy [95], was seen in grade I secondary peripheral chondrosarcomas.

Further progression towards high-grade secondary peripheral chondrosarcomas is characterised by polyploidisation, which is thought to be evolved from near-haploid precursor clones [89], and overexpression of p53 [95].

Near-haploidy was not found in osteochondromas [87, 88] or in high grade peripheral chondrosarcomas [95] and can be considered a progression marker towards a low malignant phenotype [89].

## Patient management

### Diagnosis

With the identification of *EXT1* and *EXT2* as the genes causative of Multiple Osteochondromas, it has become possible to screen patients with multiple lesions for germline mutations in either *EXT* gene in a diagnostic setting. However this procedure is time consuming and costly and therefore it is important to select patients carefully on the basis of family history, radiologic documentation and, if available, review of histology of resected lesions.

The diagnosis of Multiple Osteochondromas is based on the combination of two or more radiologically documented osteochondromas originating from the juxta-metaphyseal region of the long bones [2, 4], with or without a positive family history. Radiologically, Multiple Osteochondroma patients have a typical phenotype, easy to recognise by the expert eye. This can exclude the differential diagnoses of other skeletal disorders like metachondromatosis [100, 101], dysplasia epiphysealis hemimelica [102, 103] or non-hereditary syndromes that occur in multiple bones such as enchondromatosis (Ollier's disease) [102, 104]. Given the specific radiologic and histological expertise needed, it is recommended to seek for an expert opinion from a bone tumour specialist or from a national bone tumour registry consisting of clinicians, radiologists and pathologists, before screening for germline mutations.

If the typical Multiple Osteochondroma radiologic phenotype is present, it is important to evaluate the patient's family history to see whether other relatives are (possibly) affected. From these family members radiologic studies and, if available, histology of resected lesions can be examined. If there are other affected family members, Multiple Osteochondromas can be clinically established.

Then subsequent *EXT* mutation analysis is optional. However it can be useful to screen for germline mutations

in family members presenting a mild or no phenotype and this will also give insight into the inheritance pattern (penetrance) of the specific mutation. A known *EXT* mutation can also be used for prenatal diagnostics. If there is no positive family history, Multiple Osteochondromas cannot be excluded, since it is possible that the patient is the founder of a new Multiple Osteochondroma family and these index patients should be screened for *EXT* mutations.

Mutation analysis for *EXT1* and *EXT2* can be performed on peripheral blood of the patient. This can be established through PCR and subsequent sequencing of all exons of *EXT1* and *EXT2* [30] and/or two-colour multiplex ligation-dependent probe amplification (MLPA) [105]. When a mutation in either gene is found, the Multiple Osteochondromas diagnosis can be confirmed. If there is no mutation the diagnosis of Multiple Osteochondromas cannot be excluded, since there is a small possibility that the mutation could not be detected due to technical limitations. With the currently used methods it is possible to detect point mutations or gross deletions in 75-88% of the Multiple Osteochondroma patients [105]. These methods cannot detect positional changes, like translocations, inversions, insertions or transpositions. These changes affect the structure of the gene without changing the sequence or dosage of exons.

### Follow-up

When the diagnosis of Multiple Osteochondromas is established, patients should have a regular follow-up to discover potential malignant transformation at an early stage and enable adequate treatment to be implemented. To our knowledge, the literature does not mention a specific clinical and/or radiologic consensus about the most proper method for the follow-up of patients with proven Multiple Osteochondromas. The following pathways for both clinical and radiologic follow-up can be followed. Localisation of all, relatively larger, osteochondromas can be established with a base-line bone scan, which shows increased bone activity within the skeleton at sites of increased bone turnover, like at the sites of osteochondromas, but also at the epiphysis and apophyses of growing bones. Since secondary peripheral chondrosarcomas are extremely rare before puberty, this is, therefore, only recommended for patients who have reached skeletal maturation. Regular follow-up before that time is not necessary unless the patient presents with clinical complaints. A number of osteochondromas will demonstrate a normal uptake of the radiopharmakon, demonstrating complete maturation, while others may still show an increased activity of the radiopharmakon. This finding, at the base-line, does not immediately and specifically imply malignant transformation, but can well be explained by,

## DIAGNOSIS

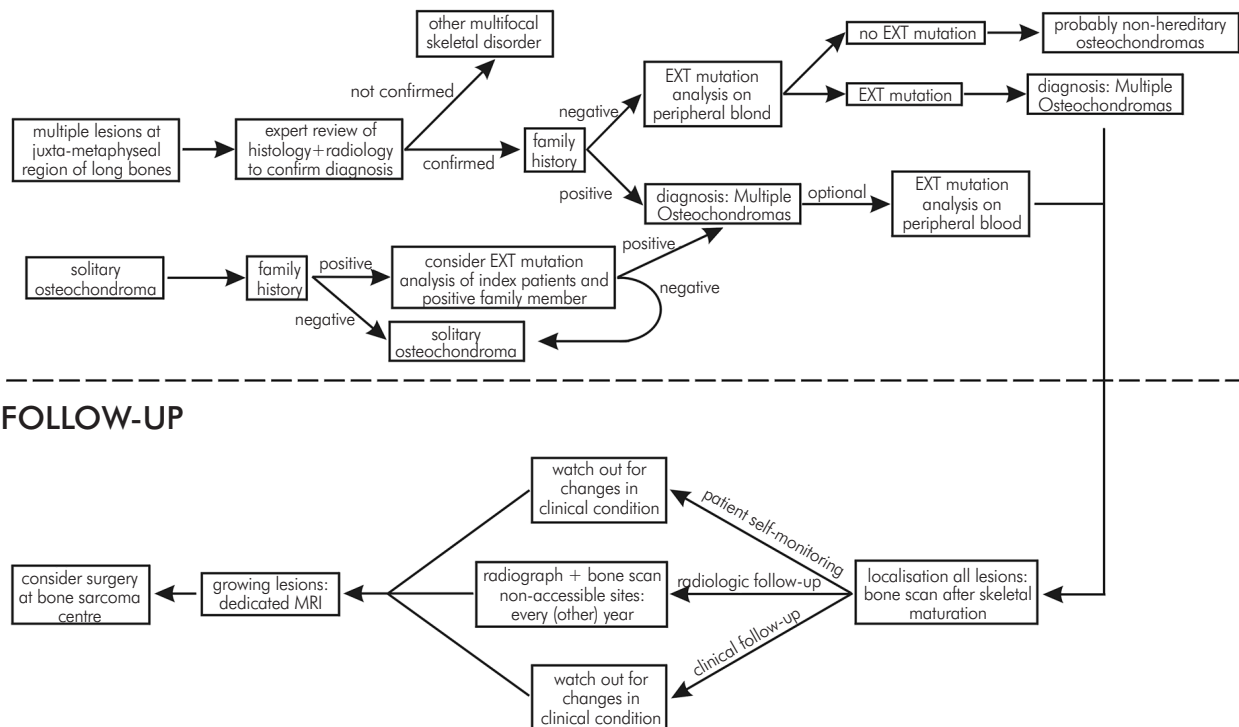


Fig. 7. Overview of systematic steps to screen and follow-up (suspected) Multiple Osteochondroma patients

as yet, incomplete maturation of the osteochondroma or just by its distinct size. Furthermore, base-line plain radiographic examinations of areas that are not accessible to palpation, like the chest, pelvis and scapula are recommended, because in these areas of the body late detection of malignant transformation of an osteochondroma towards peripheral chondrosarcoma is most common.

After these base-line examinations, patients with Multiple Osteochondromas could routinely be seen, each year or every two years, in the outpatient clinic for clinical and radiologic follow-up. It should be emphasised to the patients to come at an earlier time if changes in their clinical condition occur, such as pain or growth of a known lesion. It is also important to realise that no new osteochondromas develop after skeletal maturation.

Radiologic follow-up could consist of both plain radiographs of the pelvis, chest and scapulae in combination with follow-up bone scans. Changes in the clinical history and findings, in combination with changes on the plain radiographs or bone scans, should be regarded with suspicion. As to changes in the uptake of the radiopharmakon on bone scans however, it should be considered that increase of the

uptake does not always indicate malignant transformation. It can also be a result of trauma or the formation of an overlying bursa or inflammatory reaction. Nevertheless, these changes warrant further examination through plain radiographs and dedicated magnetic resonance (MR) imaging, including contrast-enhanced MR sequences. Also the thickness of the cartilage cap can be monitored with MR imaging.

Radiologic skeletal surveys, as a means of follow-up, do not seem to be of additional value. The role of ultrasound, in the follow-up of lesions, is still controversial and needs further studies.

The entire purpose of adequate follow-up is aimed at the early detection of malignant transformation, which enables adequate surgical treatment consisting of en-bloc resection of the lesion and its pseudocapsule with tumour-free margins, preferably in an oncology centre with experience in treating bone sarcomas. Inadequate primary surgery of a secondary peripheral chondrosarcoma will inevitably result in recurrences and can eventually result in the death caused by local problems or even metastases.

The process of making a Multiple Osteochondroma diagnosis and patient follow-up is summarized in a flowchart (Fig. 7).

## Conclusion

With all new developments and discoveries in the genetic, pathological and radiologic behaviour of osteochondromas and secondary peripheral chondrosarcomas, it has become possible to screen and carefully monitor Multiple Osteochondroma patients and their families. This will enable us to provide patients with more adequate care and treatment strategies.

## References

- Mulder JD, Schütte HE, Kroon HM and Taconis WK. Radiologic Atlas of Bone Tumors. 2 Ed., Elsevier, Amsterdam 1993.
- Bovee JVMG and Hogendoorn PCW. Multiple osteochondromas. In: Fletcher CDM, Unni KK and Mertens F (eds). World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of Soft Tissue and Bone. IARC Press, Lyon 2002.
- Dahlin's Bone Tumors General Aspects and Data on 11,087 Cases. 5th Ed., Lippincott-Raven Publishers, Philadelphia 1996.
- Legeai-Mallet L, Munnich A, Maroteaux P and Le Merrer M. Incomplete penetrance and expressivity skewing in hereditary multiple exostoses. *Clin Genet* 1997; 52: 12-16.
- Schmale GA, Conrad EU and Raskind WH. The natural history of hereditary multiple exostoses. *J Bone Joint Surg [Am]* 1994; 76A: 986-992.
- Wicklund LC, Pauli RM, Johnston D and Hecht JT. Natural history study of hereditary multiple exostoses. *Am J Med Genet* 1995; 55: 43-46.
- Khurana J, Abdul-Karim F and Bovee JVMG. Osteochondroma. In: Fletcher CDM, Unni KK and Mertens F (eds). World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of Soft Tissue and Bone. IARC Press, Lyon 2002.
- Huvos AG. Bone tumors. Diagnosis, treatment, and prognosis. 2 Ed., W.B. Saunders Company, Philadelphia 1991.
- Cooper A. Exostosis. In: Cooper A and Travers B (eds). Surgical Essays. Cox&Son, London 1818.
- Crandall BF, Field LL, Sparkes RS and Spence MA. Hereditary multiple exostoses; report of a family. *Clin Orthop* 1983; 190: 217-219.
- Boyer A. Traite des Maladies Chirurgicales. Ve. Migneret, Paris 1814.
- Shapiro F, Simon S and Glimcher MJ. Hereditary multiple exostoses. Anthropometric, roentgenographic, and clinical aspects. *J Bone Joint Surg Am* 1979; 61 (6A): 815-824.
- Willms R, Hartwig C-H, Böhm P and Sell S. Malignant transformation of a multiple cartilaginous exostosis - a case report. *Int Orthop* 1997; 21: 133-136.
- Springfield DS, Gebhardt MC and McGuire MH. Chondrosarcoma: a review. *J Bone Joint Surg [Am]* 1996; 78A: 141-149.
- Bertoni F, Bacchini P and Hogendoorn PCW. Chondrosarcoma. In: Fletcher CDM, Unni KK and Mertens F (eds). World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of Soft Tissue and Bone. IARC Press, Lyon 2002.
- Geirnaerd MJ, Bloem JL, Eulderink F, Hogendoorn PC and Taminiau AH. Cartilaginous tumors: correlation of gadolinium-enhanced MR imaging and histopathologic findings. *Radiology* 1993; 186 (3): 813-817.
- Geirnaerd MJ, Hogendoorn PC, Bloem JL, Taminiau AH and Van der Woude HJ. Cartilaginous tumors: fast contrast-enhanced MR imaging. *Radiology* 2000; 214 (2): 539-546.
- Evans HL, Ayala AG and Romsdahl MM. Prognostic factors in chondrosarcoma of bone. A clinicopathologic analysis with emphasis on histologic grading. *Cancer* 1977; 40: 818-831.
- Lamovec J, Spiler M and Jevic V. Osteosarcoma arising in a solitary osteochondroma of the fibula. *Arch Pathol Lab Med* 1999; 123 (9): 832-834.
- Matsuno T, Ichioka Y, Yagi T and Ishii S. Spindle-cell sarcoma in patients who have osteochondromatosis. A report of two cases. *J Bone Joint Surg [Am]* 1988; 70: 137-141.
- Bovee JVMG, Sakkers RJB, Geirnaerd MJA and Taminiau AHM. Intermediate grade osteosarcoma and chondrosarcoma arising in an osteochondroma. A case report of a patient with hereditary multiple exostoses. *J Clin Pathol* 2002; 55: 226-229.
- Tschiya H, Morikawa S and Tomita K. Osteosarcoma arising from a multiple exostoses lesion: case report. *Jpn J Clin Oncol* 1990; 20: 296-298.
- Ahn J, Ludecke H-J, Lindow S, Horton WA, Lee B, Wagner MJ, Horsthemke B and Wells DE. Cloning of the putative tumour suppressor gene for hereditary multiple exostoses (EXT1). *Nature Genet* 1995; 11: 137-143.
- Wuyts W, Van Hul W, Wauters J, Nemtsova M, Reyniers E, Van Hul EV, De Boule K, de Vries BB, Hendrickx J, Herrygers I, Bossuyt P, Balemans W, Fransens E, Vits L, Coucke P, Nowak NJ, Shows TB, Mallet L, van den Ouweland AM, McGaughan J, Halley DJ and Willems PJ. Positional cloning of a gene involved in hereditary multiple exostoses. *Hum Mol Genet* 1996; 5 (10): 1547-1557.
- Stickens D, Clines G, Burbee D, Ramos P, Thomas S, Hogue D, Hecht JT, Lovett M and Evans GA. The EXT2 multiple exostoses gene defines a family of putative tumour suppressor genes. *Nature Genet* 1996; 14: 25-32.
- Raskind WH, Conrad EU III, Matsushita M, Wijsman EM, Wells DE, Chapman N, Sandell LJ, Wagner M and Houck J. Evaluation of locus heterogeneity and EXT1 mutations in 34 families with hereditary multiple exostoses. *Hum Mutat* 1998; 11 (3): 231-239.
- Legeai-Mallet L, Marguerite-Jeannin P, Lemdani M, Le Merrer M, Plauchu H, Maroteaux P, Munnich A and Clerget-Darpoux F. An extension of the admixture test for the study of genetic heterogeneity in hereditary multiple exostoses. *Hum Genet* 1997; 99: 298-302.
- Philippe C, Porter DE, Emerton ME, Wells DE, Simpson AH and Monaco AP. Mutation screening of the EXT1 and EXT2 genes in patients with hereditary multiple exostoses. *Am J Hum Genet* 1997; 61: 520-528.
- Hecht JT, Hogue D, Wang Y, Blanton SH, Wagner M, Strong LC, Raskind W, Hansen MF and Wells D. Hereditary multiple exostoses (EXT): mutational studies of familial EXT1 cases and EXT-associated malignancies. *Am J Hum Genet* 1997; 60: 80-86.
- Wuyts W, Van Hul W, De Boule K, Hendrickx J, Bakker E, Vanhoenacker F, Mollica F, Ludecke HJ, Sayli BS, Pazzaglia UE, Mortier G, Hamel B, Conrad EU, Matsushita M, Raskind WH and Willems PJ. Mutations in the EXT1 and EXT2 genes in hereditary multiple exostoses. *Am J Hum Genet* 1998; 62: 346-354.
- Bovee JVMG, Cleton-Jansen AM, Wuyts W, Caethoven G, Taminiau AHM, Bakker E, Van Hul W, Cornelisse CJ and Hogendoorn PC. EXT-mutation analysis and loss of heterozygosity in sporadic and hereditary osteochondromas and secondary chondrosarcomas. *Am J Hum Genet* 1999; 65 (3): 689-698.
- Xu L, Xia J, Jiang H, Zhou J, Li H, Wang D, Pan Q, Long Z, Fan C and Deng HX. Mutation analysis of hereditary multiple exostoses in the Chinese. *Hum Genet* 1999; 105: 45-50.
- Park KJ, Shin K-H, Ku J-L, Cho T-J, Lee SH, Choi IH, Phillippe C, Monaco AP, Porter DE and Park JG. Germline mutations in the EXT1 and EXT2 genes in Korean patients with hereditary multiple exostoses. *J Hum Genet* 1999; 44: 230-234.
- Shi YR, Wu JY, Hsu YA, Lee CC, Tsai CH and Tsai FJ. Mutation screening of the EXT genes in patients with hereditary multiple exostoses in Taiwan. *Genet Test* 2002; 6 (3): 237-243.
- Zak BM, Crawford BE and Esko JD. Hereditary multiple exostoses and heparan sulfate polymerization. *Biochim Biophys Acta* 2002; 1573 (3): 346-355.
- Knudson AG Jr. Mutation and cancer: statistical study of retinoblastoma. *Proc Natl Acad Sci USA* 1971; 68 (4): 820-823.

37. Carroll KL, Yandow SM, Ward K and Carey JC. Clinical correlation to genetic variations of hereditary multiple exostoses. *J Pediatr Orthop* 1999; 19: 785-791.
38. Francannet C, Cohen-Tanugi A, Le Merrer M, Munnich A, Bonaventure J and Legeai-Mallet L. Genotype-phenotype correlation in hereditary multiple exostoses. *J Med Genet* 2001; 38 (7): 430-434.
39. Le Merrer M, Legeai-Mallet L, Jeannin PM, Horsthemke B, Schinzel A, Plauchu H, Toutain A, Achard F, Munnich A and Maroteaux P. A gene for hereditary multiple exostoses maps to chromosome 19p. *Hum Mol Genet* 1994; 3: 717-722.
40. Wise CA, Clines GA, Massa H, Trask BJ and Lovett M. Identification and localization of the gene for EXTL, a third member of the multiple exostoses gene family. *Genome Res* 1997; 7 (1): 10-16.
41. Wuyts W, Van Hul W, Hendrickx J, Speleman F, Wauters J, De Boule K, Van Roy N, Van Agtmael T, Bossuyt P and Willems PJ. Identification and characterization of a novel member of the EXT gene family, EXTL2. *Eur J Hum Genet* 1997; 5: 382-389.
42. Van Hul W, Wuyts W, Hendrickx J, Speleman F, Wauters J, De Boule K, Van Roy N, Bossuyt P and Willems PJ. Identification of a third EXT-like gene (EXTL3) belonging to the EXT gene family. *Genomics* 1998; 47: 230-237.
43. Arai T, Akiyama Y, Nagasaki H, Murase N, Okabe S, Ikeuchi T, Saito K, Iwai T and Yuasa Y. EXTL3/EXTR1 alterations in colorectal cancer cell lines. *Int J Oncol* 1999; 15 (5): 915-919.
44. Hall BD, Langer LO, Giedion A, Smith DW, Cohen MM Jr., Beals RK and Brandner M. Langer-Giedion syndrome. *Birth Defects Orig Artic Ser* 1974; 10 (12): 147-164.
45. Ludecke H-J, Johnson C, Wagner MJ, Wells DE, Turleau C, Tommerup N, Latos-Bielenska A, Sandig KR, Meinecke P, Zabel B and Horsthemke B. Molecular definition of the shortest region of deletion overlap in the Langer-Giedion syndrome. *Am J Hum Genet* 1991; 49: 1197-1206.
46. Cook A, Raskind W, Blanton SH, Pauli RM, Gregg RG, Francomano CA, Puffenberger E, Conrad EU, Schmale G, Schellenberg G, Wijsman E, Hecht JT, Wells D and Wagner MJ. Genetic heterogeneity in families with hereditary multiple exostoses. *Am J Hum Genet* 1993; 53: 71-79.
47. Ludecke H-J, Ahn J, Lin X, Hill A, Wagner MJ, Schomburg L, Horsthemke B and Wells DE. Genomic organization and promoter structure of the human EXT1 gene. *Genomics* 1997; 40: 351-354.
48. Lohmann DR, Buiting K, Ludecke H-J and Horsthemke B. The murine Ext1 gene shows a high level of sequence similarity with its human homologue and is part of a conserved linkage group on chromosome 15. *Cytogenet Cell Genet* 1997; 76: 164-166.
49. Lin X and Wells D. Isolation of the mouse cDNA homologous to the human EXT1 gene responsible for hereditary multiple exostoses. *DNA Seq.* 1997; 7 (3-4): 199-202.
50. Bellaiche Y, The I and Perrimon N. Tout-velu is a drosophila homologue of the putative tumour suppressor EXT1 and is needed for Hh diffusion. *Nature* 1998; 394: 85-88.
51. Clines GA, Ashley JA, Shah S and Lovett M. The structure of the human multiple exostoses 2 gene and characterization of homologs in mouse and *Caenorhabditis elegans*. *Genome Res* 1997; 7: 359-367.
52. Wu Y-Q, Heutink P, De Vries BBA, Sandkuijl LA, Van den Ouweland AMW, Niermeijer MF, Galjaard H, Reyniers E, Willems PJ and Halley DJ. Assignment of a second locus for multiple exostoses to the pericentromeric region of chromosome 11. *Hum Mol Genet* 1994; 3: 167-171.
53. Wuyts W, Ramlakhan S, Van Hul W, Hecht JT, Van den Ouweland AMW, Raskind WH, Hofstede FC, Reyniers E, Wells DE, de Vries B, Conrad EU, Hill A, Zalatajev D, Weissenbach J, Wagner MJ, Bakker E, Halley DJJ and Willems PJ. Refinement of the multiple exostoses locus (EXT2) to a 3-cM interval on chromosome 11. *Am J Hum Genet* 1995; 57: 382-387.
54. Stickens D and Evans GA. Isolation and characterization of the murine homolog of the human EXT2 multiple exostoses gene. *Biochem Mol Med* 1997; 61: 16-21.
55. Han C, Belenkaya TY, Khodoun M, Tauchi M, Lin X and Lin X. Distinct and collaborative roles of Drosophila EXT family proteins in morphogen signalling and gradient formation. *Development* 2004; 131 (7): 1563-1575.
56. Potocki L and Shaffer LG. Interstitial deletion of 11(p11.2p12): a newly described contiguous gene deletion syndrome involving the gene for hereditary multiple exostoses (EXT2). *Am J Med Genet* 1996; 62: 319-325.
57. Bartsch O, Wuyts W, Van Hul W, Hecht JT, Meinecke P, Hogue D, Werner W, Zabel B, Hinkel GK, Powell CM, Shaffer LG and Willems PJ. Delineation of a contiguous gene syndrome with multiple exostoses, enlarged parietal foramina, craniofacial dysostosis, and mental retardation, caused by deletions on the short arm of chromosome 11. *Am J Hum Genet* 1996; 58: 734-742.
58. Esko JD and Selleck SB. Order out of chaos: assembly of ligand binding sites in heparan sulfate. *Annu Rev Biochem* 2002; 71: 435-471.
59. Lind T, Tufaro F, McCormick C, Lindahl U and Lidholt K. The putative tumor suppressors EXT1 and EXT2 are glycosyltransferases required for the biosynthesis of heparan sulfate. *J Biol Chem* 1998; 273 (41): 26265-26268.
60. Kitagawa H, Shimakawa H and Sugahara K. The tumor suppressor EXT-like gene EXTL2 encodes an alpha1, 4-N-acetylhexosaminyltransferase that transfers N-acetylglucosamine to the common glycosaminoglycan-protein linkage region. *J Biol Chem* 1999; 274 (20): 13933-13937.
61. McCormick C, Duncan G and Tufaro F. New perspectives on the molecular basis of hereditary bone tumours. *Mol Med Today* 1999; 5: 481-486.
62. McCormick C, Duncan G, Goutsos KT and Tufaro F. The putative tumor suppressors EXT1 and EXT2 form a stable complex that accumulates in the Golgi apparatus and catalyzes the synthesis of heparan sulfate. *Proc Natl Acad Sci USA* 2000; 97 (2): 668-673.
63. Rubin JB, Choi Y and Segal RA. Cerebellar proteoglycans regulate sonic hedgehog responses during development. *Development* 2002; 129 (9): 2223-2232.
64. Cardin AD and Weintraub HJ. Molecular modeling of proteoglycosaminoglycan interactions. *Arteriosclerosis* 1989; 9 (1): 21-32.
65. Knudson CB and Knudson W. Cartilage proteoglycans. *Semin Cell Dev Biol* 2001; 12 (2): 69-78.
66. Liu W, Litwack ED, Stanley MJ, Langford JK, Lander AD and Sanderson RD. Heparan sulfate proteoglycans as adhesive and anti-invasive molecules. Syndecans and glypican have distinct functions. *J Biol Chem* 1998; 273 (35): 22825-22832.
67. David G, Bai XM, Van der Schueren B, Marynen P, Cassiman JJ and Van den Berghe H. Spatial and temporal changes in the expression of fibroglycan (syndecan-2) during mouse embryonic development. *Development* 1993; 119 (3): 841-854.
68. Zimmermann P and David G. The syndecans, tuners of transmembrane signaling. *FASEB J* 1999; 13 Suppl:S91-S100: S91-S100.
69. Seghatolleslami MR and Kosher RA. Inhibition of in vitro limb cartilage differentiation by syndecan-3 antibodies. *Dev Dyn* 1996; 207 (1): 114-119.
70. Shimo T, Gentili C, Iwamoto M, Wu C, Koyama E and Pacifici M. Indian hedgehog and syndecans-3 coregulate chondrocyte proliferation and function during chick limb skeletogenesis. *Dev Dyn* 2004; 229 (3): 607-617.
71. Veugeliers M, De Cat B, Ceulemans H, Bruystens AM, Coomans C, Durr J, Vermeesch J, Marynen P and David G. Glypican-6, a new member of the glypican family of cell surface heparan sulfate proteoglycans. *J Biol Chem* 1999; 274 (38): 26968-26977.
72. SundarRaj N, Fite D, Ledbetter S, Chakravarti S and Hassell JR. Perlecan is a component of cartilage matrix and promotes chondrocyte attachment. *J Cell Sci* 1995; 108 (Pt 7): 2663-2672.
73. Ponta H, Sherman L and Herrlich PA. CD44: from adhesion molecules to signalling regulators. *Nat Rev Mol Cell Biol* 2003; 4 (1): 33-45.

74. van der Voort R, Taher TE, Wielenga VJ, Spaargaren M, Prevo R, Smit L, David G, Hartmann G, Gherardi E and Pals ST. Heparan sulfate-modified CD44 promotes hepatocyte growth factor/scatter factor-induced signal transduction through the receptor tyrosine kinase c-Met. *J Biol Chem* 1999; 274 (10): 6499-6506.
75. The I, Bellaiche Y and Perrimon N. Hedgehog movement is regulated through tout velu -dependant synthesis of a heparan sulfate proteoglycan. *Mol Cell* 1999; 4 (4): 633-639.
76. Bornemann DJ, Duncan JE, Staatz W, Selleck S and Warrior R. Abrogation of heparan sulfate synthesis in *Drosophila* disrupts the Wingless, Hedgehog and Decapentaplegic signaling pathways. *Development* 2004; 131 (9): 1927-1938.
77. Stickens D, Brown D and Evans GA. EXT genes are differentially expressed in bone and cartilage during mouse embryogenesis. *Dev Dyn* 2000; 218 (3): 452-464.
78. Amling M, Neff L, Tanaka S, Inoue D, Kuida K, Weir E, Philbrick WM, Broadus AE and Baron R. Bcl-2 lies downstream of parathyroid hormone related peptide in a signalling pathway that regulates chondrocyte maturation during skeletal development. *J Cell Biol* 1997; 136: 205-213.
79. Van der Eerden BCJ, Karperien M, Gevers EF, Lowik CWGM and Wit JM. Expression of Indian Hedgehog, PTHrP and their receptors in the postnatal growth plate of the rat: evidence for a locally acting growth restraining feedback loop after birth. *J Bone Miner Res* 2000; 15 (6): 1045-1055.
80. Erlebacher A, Filvaroff EH, Gitelman SE and Derynck R. Toward a molecular understanding of skeletal development. *Cell* 1995; 80: 371-378.
81. Goldfarb M. Functions of fibroblast growth factors in vertebrate development. *Cytokine and Growth Factor Reviews* 1996; 7 (4): 311-325.
82. Kato Y and Iwamoto M. Fibroblast growth factor is an inhibitor of chondrocyte terminal differentiation. *J Biol Chem* 1990; 265 (10): 5903-5909.
83. Iwamoto M, Shimazu A, Nakashima K, Suzuki F and Kato Y. Reduction of basic fibroblasts growth factor receptor is coupled with terminal differentiation of chondrocytes. *J Biol Chem* 1991; 266 (1): 461-467.
84. Liu Z, Xu J, Colvin JS and Ornitz DM. Coordination of chondrogenesis and osteogenesis by fibroblast growth factor 18. *Genes Dev* 2002; 16 (7): 859-869.
85. Sahni M, Ambrosetti D-C, Mansukhani A, Gertner R, Levy D and Basilico C. FGF signaling inhibits chondrocyte proliferation and regulates bone development through the STAT-1 pathway. *Genes Dev* 1999; 13: 1361-1366.
86. Naski MC, Colvin JS, Coffin JD and Ornitz DM. Repression of hedgehog signaling and BMP4 expression in growth plate cartilage by fibroblast growth factor receptor 3. *Development* 1998; 125: 4977-4988.
87. Bridge JA, Nelson M, Orndal C, Bhatia P and Neff JR. Clonal karyotypic abnormalities of the hereditary multiple exostoses chromosomal loci 8q24.1 (EXT1) and 11p11-12 (EXT2) in patients with sporadic and hereditary osteochondromas. *Cancer* 1998; 82: 1657-1663.
88. Mertens F, Rydholm A, Kreicbergs A, Willen H, Jonsson K, Heim S, Mitelman F and Mandahl N. Loss of chromosome band 8q24 in sporadic osteochondromas. *Genes Chromosomes Cancer* 1994; 9: 8-12.
89. Bovee JVMG, Royen MV, Bardeel AFJ, Rosenberg C, Cornelisse CJ, Cleton-Jansen AM and Hogendoorn PC. Near-haploidy and subsequent polyploidization characterize the progression of peripheral chondrosarcoma. *Am J Pathol* 2000; 157 (5): 1587-1595.
90. Lin X, Wei G, Shi Z, Dryer L, Esko JD, Wells DE, Matzuk MM. Disruption of gastrulation and heparan sulfate biosynthesis in EXT1-deficient mice. *Dev Biol* 2000; 224 (2): 299-311.
91. Koziel L, Kunath M, Kelly OG and Vortkamp A. Ext1-dependent heparan sulfate regulates the range of Ihh signaling during endochondral ossification. *Dev Cell* 2004; 6 (6): 801-813.
92. Hecht JT, Hall CR, Snuggs M, Hayes E, Haynes R and Cole WG. Heparan sulfate abnormalities in exostosis growth plates. *Bone* 2002; 31 (1): 199-204.
93. Yamada S, Busse M, Ueno M, Kelly OG, Skarnes WC, Sugahara K and Kusche-Gullberg M. Embryonic fibroblasts with a gene trap mutation in EXT1 produce short heparan sulphate chains. *J Biol Chem* 2004; 279 (31): 32134-32141.
94. Bovee JVMG, Van den Broek LJC, Cleton-Jansen AM and Hogendoorn PCW. Up-regulation of PTHrP and Bcl-2 expression characterizes the progression of osteochondroma towards peripheral chondrosarcoma and is a late event in central chondrosarcoma. *Lab Invest* 2000; 80: 1925-1933.
95. Bovee JVMG, Cleton-Jansen AM, Kuipers-Dijkshoorn N, Van den Broek LJC, Taminiau AHM, Cornelisse CJ and Hogendoorn PC. Loss of heterozygosity and DNA ploidy point to a diverging genetic mechanism in the origin of peripheral and central chondrosarcoma. *Genes Chromosomes Cancer* 1999; 26: 237-246.
96. Bovee JVMG, Sciort R, Cin PD, Debiec-Rychter M, Zelderen-Bhola SL, Cornelisse CJ and Hogendoorn PC. Chromosome 9 alterations and trisomy 22 in central chondrosarcoma: a cytogenetic and DNA flow cytometric analysis of chondrosarcoma subtypes. *Diagn Mol Pathol* 2001; 10 (4): 228-235.
97. Xiang JH, Spanier SS, Benson NA and Braylan RC. Flow cytometric analysis of DNA in bone and soft-tissue tumors using nuclear suspensions. *Cancer* 1987; 59: 1951-1958.
98. Helio H, Karaharju E and Nordling S. Flow cytometric determination of DNA content in malignant and benign bone tumours. *Cytometry* 1985; 6: 165-171.
99. Mandahl N, Baldetorp B, Ferno M, Akerman M, Rydholm A, Heim S, Willen H, Killander D and Mitelman F. Comparative cytogenetic and DNA flow cytometric analysis of 150 bone and soft-tissue tumors. *Int J Cancer* 1993; 53: 358-364.
100. Bassett GS and Cowell HR. Metachondromatosis. Report of four cases. *J Bone Joint Surg Am* 1985; 67 (5): 811-814.
101. Maroteaux P. Metachondromatosis. *Z Kinderheilkd* 1971; 109 (3): 246-261.
102. Murphey MD, Flemming DJ, Boyea SR, Bojeskul JA, Sweet DE and Temple HT. From the archives of the AFIP Enchondroma versus chondrosarcoma in the appendicular skeleton: differentiating features. *Radiographics* 1998; 18 (5): 1213-1237.
103. Fairbank TJ. Dysplasia epiphysialis hemimelica (tarso-epiphysial acclasis). *J Bone Joint Surg Br* 1956; 38-B (1): 237-257.
104. Ollier M. Dyschondroplasia. *Lyon Med* 1900; 93: 23-25.
105. White SJ, Vink GR, Kriek M, Wuyts W, Schouten J, Bakker B, Breuning MH and den Dunnen JT. Two-color multiplex ligation-dependent probe amplification: detecting genomic rearrangements in hereditary multiple exostoses. *Hum Mutat* 2004; 24 (1): 86-92.
106. Stenson PD, Ball EV, Mort M, Phillips AD, Shiel JA, Thomas NS, Abeyasinghe S, Krawczak M and Cooper DN. Human Gene Mutation Database (HGMD): 2003 update. *Hum Mutat* 2003; 21 (6): 577-581.
107. Kim BT, Kitagawa H, Tamura J, Saito T, Kusche-Gullberg M, Lindahl U and Sugahara K. Human tumor suppressor EXT gene family members EXTL1 and EXTL3 encode alpha 1,4- N-acetylglucosaminyltransferases that likely are involved in heparan sulfate/heparin biosynthesis. *Proc Natl Acad Sci USA* 2001; 98 (13): 7176-7181.
108. Esko JD and Lindahl U. Molecular diversity of heparan sulfate. *J Clin Invest* 2001; 108 (2): 169-173.
109. Couchman JR. Syndecans: proteoglycan regulators of cell-surface microdomains? *Nat Rev Mol Cell Biol* 2003; 4 (12): 926-937.
110. Nybakken K and Perrimon N. Heparan sulfate proteoglycan modulation of developmental signaling in *Drosophila*. *Biochim Biophys Acta* 2002; 1573 (3): 280-291.